Enhanced mechanical properties of hydroxyapatite bone graft coated with silane modified gelatin

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INTRODUCTION:
Synthetic bone graft such as hydroxyapatite[Ca$_5$(PO$_4$)$_3$(OH)$_2$] (HA) ceramic have been exhibited high tissue compatibility, osteoconductivity and resistance to infection. However, the insufficient bending strength of HA ceramic, probably too tight to bind of BMPs to HA limit its usage[1]. Various hydroxyapatite/collagen composites were developed as potential biomaterials for bone substitutes due to their compositional analogy to bone. However, the problem of brittleness of ceramic is not overcome by simple collagen or gelatin coating/blending. We have developed a novel material MGSF (modified gelatin crosslinked with silane and fructose) which exhibits superior properties over gelatin or crosslinked glutaraldehyde-gelatin with improved tensile strength, stable thermodynamic property and slow degradation by a proteolytic enzyme and good cytocompatibility. The aim of this study was to characterize MGSF coated HA surface on cellular supporting, growth factor association and dissociation and to evaluate mechanical properties due to surface coating.

METHODS:
Preparation of samples: This research used the silane (Dow-Corning); Z-6040 and Z-6011. Gelatin was purchased from Aldrich-SIGMA. Briefly, in 100ml of 5% w/v gelatin solution (pH 3.85), fructose (Calbiochem) and silanes were added to make 0.5% (w/v) fructose and 3% Z-6040 and 2% v/v Z-6011. The solution was mechanically stirred at 60˚C for 10 min. The HA granule or block (ProOsteon 500R) was added and the solution was heated to over 90˚C for 10 min. Solution was kept at 65˚C for 30 min to eliminate by-product. MGSF-HA was molded and dried under vacuum for 30 min.

Mechanical Test: Test samples (cylinder with 9.84 mm long and 8.52mm diameter, n=5) were tested at dry condition and wet condition, in which samples were immersed in deionized water overnight. All tests were conducted using Instron at loading rate of 5mm/min[2].

Uniformity and thickness of MGSF coating on HA granule or blocks were immersed in 1% (w/v) NHD (Sondell Scientific Instrument) solution for 30 min. After rinsed extensively with water, blocks were embedded in PMMA resin (Sigam) to fill the void volume. Micrographs of cross-sections were observed under binocular stereo microscope (OM444TC).

Cell viability on the scaffolds of MGSF-HA: MGSF-HA granules were added to the 24-well plates including C2C12 cells with the density of 100k cells/well in 10% FBS/DMEM, followed by incubation at 37˚C with 5% CO$_2$. The medium was changed every two days. After 5 days incubation, the medium was removed from plates and petri dishes, followed by cell staining with mitochondrial tracker (MitoTracker Red CMXROS).

BMP-2 release kinetics on HA or MGSF-HA: Three hundred mg of HA and MGSF-HA granules were immersed into 150ug/ml BMP-2 solution for 6 hours. After 3 times washes with ddH$_2$O, granules were replaced in 1.0ml ddH$_2$O. At decided time interval, BMP-2 released from granule in solution was tested by C2C12 [3].

Intramuscular implantation: HA or MGSF-HA was sterilized with 70% alcohol for 20 min before implantation in rats (Fischer 344). All animal’s handling and protocols were approved by IACUC of the University of Southern California. On each 150ug MGSF coated HA granules, 4ug of BMP-2 was added before implantation and placed in thigh muscle of hind legs for 4 weeks(n=4). Samples was retrieved for histological analysis and ALP activity test.

RESULTS
MGSF coating on HA was characterized by uniformity and thickness test [Fig.1]. When stained with ninhydrin solution, the surface of untreated HA was colorless, while MGSF coated surface was changed to blue-purple color resulting in ninhydrin chromophore combined with amine groups of MGSF. MGSF-HA formed an even coated surface, about 10-20µm, in the inter pores of HA block without clogging the pores. Cells labeled with red fluorescent marker in Figure 1 were firmly attached and proliferated on the inner surface of MGSF-HA. MGSF-HA provided cell favorable attaching surface. Compressive strength of HA and MGSF-HA tested in dry and wet conditions were displayed Fig. 1- (c). All samples showed a similar stress-strain behavior, that stress was increased sharply up to the maximal loading before fracture. MGSF-HA responded efficiently to an applied stress compared to HA which failed at low loading. Compressive strength of MGSF-HA was increased ~2.5 times higher than that of HA at dry state or wet state, while elongation of MGSF-HA was similar to that of HA.

Figure 2. ALP activity test from BMP-2 released from HA or MGSF-HA. *p<0.01 with respect to HA Bar=means±SD

C2C12 ALP induced by accumulated release of BMP-2 is shown in Figure 2. MGSF-HA scaffolds released more BMP-2 than HA alone. In the first 3 hours, BMP-2 release fast and reached to steady state after 12 hours. BMP-2 seems to bind tightly to HA and release only half content at all testing periods.

Figure 3. (a) ALP activity with retrieved HA and MGSF-HA (b) Histological evaluation of implantation of MGSF-HA

Bone formation potential of MGSF-HA incorporated with 4.0 ug BMP-2 was tested ectopically in rat. ALP activity test showed there was no significantly difference in bone formation between HA alone and MGSF-HA. New bone was formed inside the pore surface of MGSF-HA [Fig. 3].

DISCUSSION
A thin layer of MGSF uniformly coated on porous HA surface significantly improves compressive strength of HA. The presence of MGSF in HA promotes the dissociation of growth factor from matrix and supports cell attachment, proliferation and differentiation. MGSF-HA bone graft inducing new bone formation with the addition of BMP-2 was similar to that of HA. These results suggest that MGSF-HA can be applied to moderate-high weight bearing sites for bone grafting.

REFERENCE:

Poster No. 1345 • 55th Annual Meeting of the Orthopaedic Research Society